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This report summarizes our initial attempts at addressing some critical questions about the fundamental function and potential applications of hybrid peptide-nanosensors. Peptides represent a happy medium between tapping into the chemical diversity of amino acids – nature's preferred recognition molecules – but without the stability issues associated with proteins. Yet, a significant number of challenges remain, including: 1) identifying larger classes of peptide binders to a host of chemical and biological targets, 2) understanding the mechanisms by which biomolecular interactions between analytes and peptides induce subsequent responses in the nano-transducers, 3) scaling these results from the few-device level to large arrays which can address many targets in parallel, and 4) translating these results from well-controlled laboratory environments to clinical applications and settings. Taken together, these results serve as a model platform in the use of peptide nanosensors for applications ranging from non-invasive breath monitoring to food spoilage or biological and chemical threat detectors.

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**Final Technical Report:**

**Grant/Contract Title: (YIP-09) BIOMIMETIC NANOSENSOR ARRAYS FOR SELECTIVE SMALL MOLECULE DETECTION**

**Grant/Contract Number: FA9550-09-1-0096**

**PI: Prof. Michael McAlpine, Department of Mechanical Engineering, Princeton University**

## Objectives

Molecular biomimetics is an emerging field in which the tools of molecular biology and nanotechnology are synergized. One area of vital relevance is chemical and biological sensing, which if implemented on biocompatible substrates, could yield breakthroughs in implantable or wearable monitoring systems. Biomimicking smart materials which integrate chemical recognition moieties with sensitive transducers could provide a general platform for highly specific analyte sensors. Nanomaterials are particularly sensitive chemical sensors because of their high surface-to-volume ratios. Our research has focused on the bio-functionalization of nanoscale materials to yield sensors which mimic the olfactory system.[1-5]

Likewise, oligopeptides are robust substrates for the selective recognition of a variety of chemical and biological species. Our current research is focused on bio-inspired approaches to mimicking olfaction by linking peptides to nanosensors for the selective detection of biological species ranging from small molecules to pathogens. The peptide/nanowire sensors are designed, via combinatorial peptide engineering, to exhibit highly selective responses to a range of target analytes, and to detect traces of these gases from "chemically camouflaged" mixtures. We anticipate that this program will reveal fundamental understandings of the relative contributions of inter-molecular reactivity and structural identification in the function of olfactory protein receptors. Furthermore, the unique capability to tailor odorant sensing should enable transformational opportunities in environmental and medical applications.

Our objectives followed those of the original proposal submission, and consisted of an integrated experimental program, building upon our discoveries in order to:

(a) Develop a more practical strategy for hierarchical scaling of our nanowires into a large platform array, while rendering the process more parallel and reliable.

(b) Validate the surface chemistry of attachment of peptide sequences to nanomaterials, and develop schemes for more scalable peptide assembly.

(c) Test the responsiveness of the hybrid peptide-nanosensors to target analytes and analyte mixtures using literature-determined peptide sequences. Perform experimental investigations into the mechanism of response. Investigate the ability of the sensors to detect and distinguish target analytes from molecular mixtures.

(d) Develop theoretical molecular models to elucidate the contributions of reactivity and molecular structure in achieving recognition. Use the developed molecular model to enable predictive peptide sequence determination.

(e) Expand the available peptide library into a generalized target analyte determination protocol via phage display – exploiting nature for hybrid biomaterials generation.

**Accomplishments:**

The development of a universally tailorable, miniaturized sensing platform for sensitive and selective detection of a variety of biochemical analytes could stimulate transformative fundamental and technological opportunities. Nanoscale materials are extremely sensitive sensors due to their high surface-to-volume ratios. Likewise, peptides are short polymers of amino acids representing robust substrates for selective recognition due to the broad chemical diversity that can be achieved within a relatively compact size. Here we explore the possibilities of linking peptides to nanosensors for the selective detection of biochemical targets. Such systems raise a number of interesting fundamental challenges: What are the peptide sequences, and how can rational design be used to derive selective binders? What nanomaterials should be used, and what are some strategies for assembling hybrid nanosensors? What role does molecular modeling play in elucidating response mechanisms? What is the resulting performance of these sensors, in terms of sensitivity, selectivity, and response time? What are some potential biomedical applications? This report will highlight our results at addressing these research challenges. Specifically, peptide sequences that originate from nature or that are identified from phage display are used as capture elements. The sensors are based on a variety of nanomaterials, including nanowires, graphene, and carbon nanotubes. Peptide coupling is achieved via traditional surface functionalization methods or self-assembly. Molecular modeling provides detailed insights into the hybrid nanostructure as well as detection mechanisms. The peptide nanosensors are shown to distinguish chemically camouflaged mixtures of vapors and detect chemical warfare agents, with achievable sensitivity limits at parts-per-billion levels. Finally, we anticipate future uses of this technology in biomedical areas, including disease detection via molecular contents of the breath. Overall, these results provide a novel platform for what might be achieved in terms of highly sensitive and selective "nanoelectronic noses."

**A. Overview**

Molecular biomimetics is an emerging field in which the tools of molecular biology and nanotechnology are synergized. Significant research efforts have been dedicated to the synthesis and characterization of a variety of nanoscale materials with properties enhanced by finite size effects. Similarly explosive advances in biotechnology have made it possible to custom design bioinspired materials.[6,7] Due to their similar size scales, the interfacing of biomolecules and nanomaterials could be effective in signal transduction, resulting in the generation of bioelectronic hybrid sensors with implications for defense and biomedical applications. These nanosensors may not necessarily be single component elements, but via directed assembly may be organized into macroscale systems or even "systems of systems" in the same way that a single organism, a multi-cellular organism, or a group of organisms is configured. Ultimately, we envision a platform composing a parallel arrangement of technologies, whereby a given target compound is physically provided at the start of a sequence of steps, followed by some automated process for sensing material optimization, to produce a highly sensitive and selective device that can detect minute concentrations of the input compound.

Progress in the development of highly selective and sensitive sensors has recently accelerated due to increased concerns about chemical and biological threats.[8-12] While DNA and protein biomolecular sensors can exploit well-established "lock-and-key" interactions to achieve selectivity, obtaining high selectivity and sensitivity in gas phase sensors has until recently had to rely on physical (i.e. chromatographic) separation methods or spectroscopic fingerprinting techniques. However, the associated instrumentation is limited in portability, precluding the possibility of implantable or wearable sensors, and requires skilled human operators. Arrays of chemical sensors ("electronic noses") offer a promising data-rich alternative, with the potential for continuous real-time monitoring and discrimination of large families of gases. These vapor

analyzers are designed in a combinatorial fashion to mimic the olfactory system via the integration of sensor arrays and pattern recognition algorithms.[13] The sensors yield varying responses to different analytes, and the unique response patterns acquired from such arrays can be considered as chemical fingerprints to particular analytes. Ideally, such a sensor is trained on all possible analytes that it will encounter during its lifetime. Unfortunately, such a protocol is not feasible in most cases, since the set of chemicals that will be encountered is not fully known.

An alternative is to attempt more specific sensing for the deconvolution of molecular signatures from interfering gas mixtures, without requiring an external analytical filter, excessively large sensor libraries, pattern recognition algorithms, or advance calibration. This can be achieved by focusing on the unique molecular feature interactions between the sensor elements and the target gas molecules. Mammalian olfactory systems apparently use a combination of both approaches.[14] Accurate recognition requires sensors to detect a unique molecular feature of the analyte. On the other hand, generalization to unknown chemical species requires detection of features that are common across a desired class of analytes. The opposing nature of these constraints suggests that achieving both capabilities in a single device will ultimately require a hierarchical approach, similar to what occurs in biological systems.

Odorant perception in the mammalian olfactory system results from an aggregate response of intricate biochemical and electrophysiological signaling events (Fig. 1). Mammals contain ca. 1000 genes expressing for odor reception, and odor discrimination begins with molecular feature detection upon binding to receptor proteins.[14] The combinatorial input from a large population of olfactory receptors is subsequently refined by neurological signal processing and memory association to become more odor-specific. This arrangement, in conjunction with the complex processing capabilities of the brain, renders the mammalian olfactory system one of the most effective sensing structures. Indeed, most mammals can discriminate 10,000 or more distinct odors at detection levels of only a few parts-per-billion (ppb).[15]

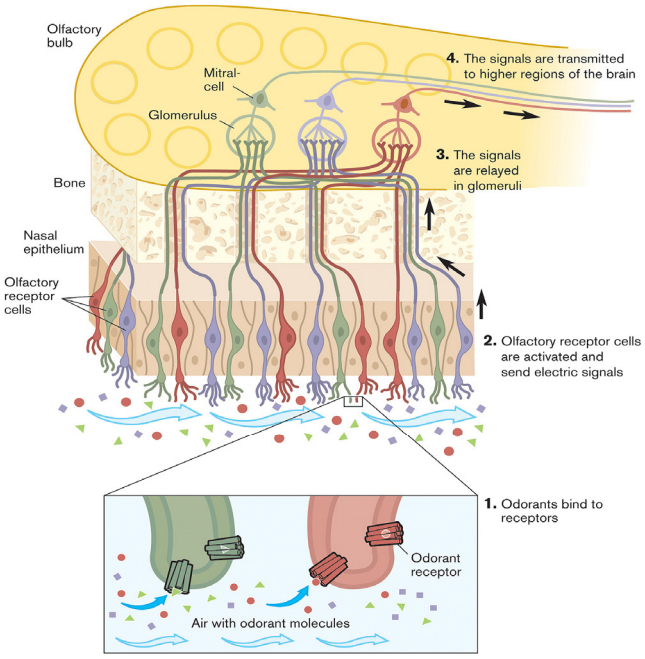


**Figure 1.** Schematic depicting odorant receptors and the pathway from chemical recognition to signal transduction in the olfactory system.

Successful attempts have been made to mimic these binding domains via the use of peptide aptamers,[16] which are biorecognition molecules that can be chemically engineered to bind specific targets. Peptides are interesting because of their broad chemical diversity (acidity, hydrophobicity, etc.) that can be achieved within a relatively compact size. Furthermore, peptides impart a degree of robustness relative to proteins and antibodies, allowing for use in more extreme environments and long-term storage. Oligopeptides have been coated onto thin film piezoelectric crystal mass sensors to achieve selectivity to various saturated vapors.[16]

Recently, electronic noses based on arrays of semiconducting nanowires[1] and nanotubes[12] have been implemented. These “nano-noses” boast ppb sensitivities, a consequence of the nanostructure diameters being comparable to the width of the surface space charge region. These nanosensors have shown some selectivity to certain molecules, via the use of chemoselective polymer coatings[17] and surface chemistry functionalizations.[1] Antibodies can also be used to impart selectivity, resulting in drastically limited sensor device lifetime. A general scheme for

achieving a high degree of specificity to a given target molecule while retaining sensor robustness is highly desirable.

Here, we describe a novel approach to achieving selectivity toward target molecules, via the interfacing of nanomaterials and peptides for the development of biomimetic nanosensors. Our results show that such hybrid sensors can display high degrees of sensitivity and selectivity toward small molecules, "chemically camouflaged" molecular mixtures, and chemical and biomedical threat agents. Through both theory and experiment, we show that the viability of our approach arises from how well chemical interactions between the peptides and specific compositional features of target analytes can be engineered. Finally, we present new techniques for nanosensor array fabrication, and combinatorial peptide selection and sequencing, in an effort to extend our approach from the single-device level to a generalized biomimicking hierarchy.

**B. Identification of Peptide Sequences**

Identifying peptide sequences which bind to targets of interest is a critically important first step in sensor development. In a previous study, thin film sensors were rendered selective to various saturated vapors using peptide sequences that were rationally designed to mimic the putative binding sites of a human olfactory protein.[16] The protein was modeled by molecular simulation methods and then small analytes were computationally "docked" onto binding sites. In addition to these modeling methods, we have identified peptide sequences using two primary approaches: 1) phage display, and 2) naturally occurring peptides.

Combinatorial libraries provide a natural selection protocol for identifying high-affinity peptides to a range of materials.[18] In biological display technologies, a phage or bacteria is genetically engineered to display a peptide sequence on the coat protein or flagella of the cell membrane protein, respectively. A large random library of phages can be generated, each displaying a different peptide sequence. The strongly binding sequences are culled via the biopanning process, in which the randomized phage library is allowed to interact with the substrate of interest.[19] Non-binding and weakly-interacting phages are removed via successive washing cycles. The bound phages are subsequently eluted from the surface, and replicated by reinfecting the host bacteria (*E. coli*). Finally, individual clones are sequenced to extract the amino acid sequence of the polypeptides binding to the target substrate material (Fig. 2). The phage display approach has been used with considerable success in mining peptide sequences with high affinities to a host of biomolecular ligands and inorganic surfaces.[20,21] We have also utilized phage display for identifying peptide binders to small molecular inks.[3]

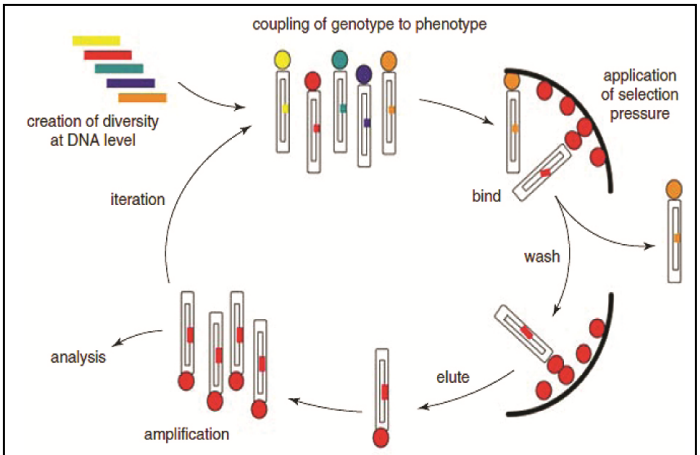


**Figure 2.** Schematic of the phage display cycle. Diversity created at the DNA level is translated into phenotypic diversity by the display of polypeptides on the phage surface. The application of selection pressure, washing, and elution, allows the selection of ligand-binding peptides.

Another approach is to exploit peptide sequences that have been developed in nature via millions of years of evolution. In insects, odor sensing occurs in the antenna. Small proteins called odorant binding proteins (OBPs) bind and ferry hydrophobic odorant molecules from the external environment to receptors on the antennal olfactory sensilla. The exceptional ability of insects to detect chemical signatures has led to the use of trained bees in a handheld device by Inscentinel™ for the detection of explosives such as trinitrotoluene (TNT). The antennal-specific

protein-1 (ASP1), an OBP from honeybee, *Apis mellifera*, contains a C-terminal tail fragment that has been shown to bind to pheromones and other chemical targets.[22] Four amino acid residues (Trp-Phe-Val-Ile, ASP1C) at the C-terminus play important roles in binding to TNT.[23] Once viable peptide sequences are identified, the next step is to develop strategies for the assembly of these peptides onto nanomaterials.

### C. Assembly of Hybrid Peptide-Nanomaterials

A key step in realizing highly sensitive and selective nanoelectronic noses is the generation of hybrid sensors via the linking of peptides to nanomaterial surfaces (Fig. 3a). We have explored two general approaches for this synergizing: 1) covalent coupling chemistry, and 2) peptide self-assembly. The choice of which approach to use is entirely dependent on the unique surface chemistry and structural integrity of the nanomaterial host.

In the case of silicon nanowires (SiNWs), the surfaces terminate in intrinsic silica, which has an established chemistry[9] that permits NW surface modification without strongly affecting the semiconducting core. SiNW arrays can be produced either from the bottom up, via VLS growth followed by fluidic assembly, or by top-down patterning methods. For biocompatible sensing applications, SiNWs can also be assembled on flexible plastic substrates.[1] Following nanowire synthesis or fabrication, sensor devices are fashioned via conventional microfabrication techniques. Bare SiNW sensors are capable of detecting ppb levels of $NO_2$, even on plastic substrates.[1]

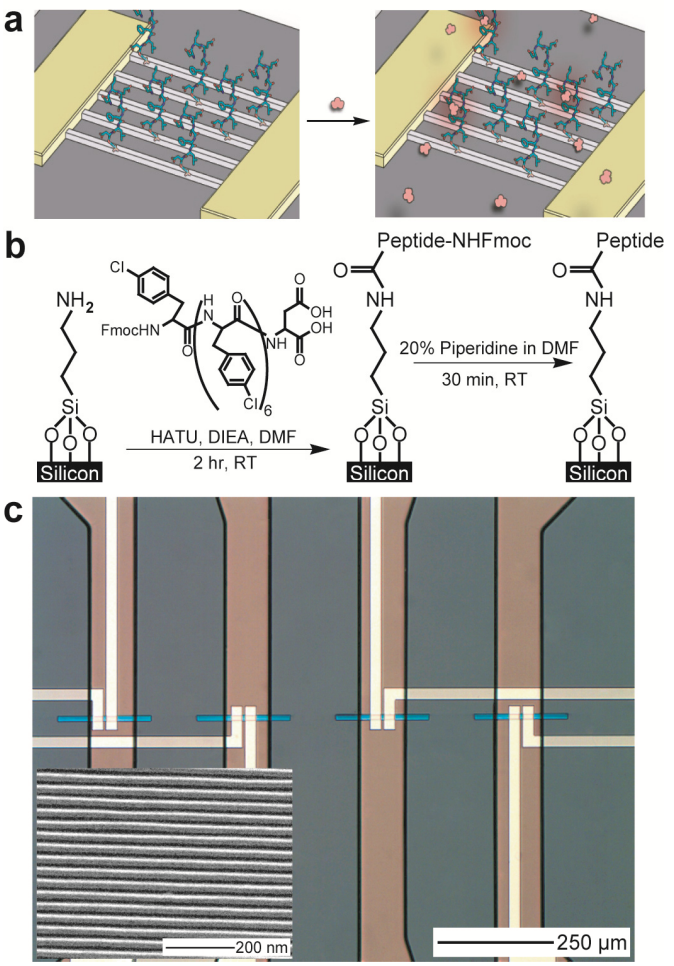


**Figure 3.** (a) Cartoon Cartoon depicting selective detection of target analyte (pink) by peptide sequences (blue/green) coupled to nanowire sensors (white). (b) Covalent Attachment of Peptides to SiNWs. (c) Optical image of microfluidic functionalization channels intersecting nanowire sensor devices. (Inset) SEM image of the SiNW film.

Peptides were immobilized onto SiNWs using amide coupling (Fig. 3b). First, the nanowire surfaces were chemically modified by immersion of the chip in an amino-silane (APTES) modifying reagent. Next, oligopeptides were synthesized with the desired recognition sequences, plus an aspartic acid "linking residue" tail at the carboxy-terminus. The peptides were dissolved in DMF, mixed with coupling reagents, and immediately injected into PDMS microfluidic chambers aligned to the device islands (Fig. 3c). These microfluidic channels permit localized modification, such that diverse peptide device arrays can be produced.

In contrast to SiNWs, single-walled carbon nanotubes (SWNTs) and graphene are single-atom thick, $sp^2$ carbon-based materials with remarkable sensing properties.[10,23-26] Yet, the ability to generically tailor their chemical and biological properties has been limited by their delicate

structures. For example, covalent functionalization can trigger symmetry breakage of the graphene lattice, thereby altering its properties. Further, non-covalent chemical modification strategies may be limited in scope of applicability.

Phage display has been previously used to identify peptides which bind to SWNTs.[23,27] We have recently developed a phage display protocol for the identification of graphene-specific peptides, which were narrowed from peptides displaying high affinities toward various graphite flakes.[2] A graphene binding peptide (GBP), with the amino acid sequence EPLQLKM, was found to specifically bind to SLP30® graphite (TIMCAL, OH, surface area = 8.0 $m^2/g$). Nanopatterned graphene has recently garnered significant interest due to the ability of geometrically confined one-dimensional graphene strips to display interesting properties, such as enhanced electrical gating.[28] We generated nanoscale patterns of graphene as a means of definitively demonstrating the relative selectivity of the identified peptide sequences. Our patterning approach, termed Photolithography and Etching for Nanoscale Lithography (PENCiL), has been described elsewhere.[29]

Figure 4a shows AFM images of defined graphene nanostrip (GNS) patterns following incubation with the graphene-binding phages displaying GBP. Revealed GNSs (diameter ~ 300 nm) incubated with GBP phages show clear binding of the phages along the entire length of the GNSs. Importantly, no phage particles are seen between the graphene NWs, showing that the selective binding to the GNSs is significantly enhanced relative to the background $Si/SiO_2$ substrate. This result shows the recognition capabilities of the phage displayed peptides, even toward graphene nanostrips.

Since the majority of the SLP30® surface consists of graphene edges, an intriguing question is whether GBP is capable of selective graphene edge-binding.[30] Freshly cleaved highly ordered pyrolytic graphite (HOPG) samples were immersed in a solution containing GBP (0.5 mg/mL) for 15 min. In AFM images shown in Figure 4b, GBP primarily decorates the edges of graphene sheets, forming structures about ~1 nm in height. By contrast, a dodecameric carbon nanotube binding peptide (CBP; HSSYWYAFNNKT)[31] was also investigated. Figure 4c shows that the CBP peptide decorates the planar HOPG surface, leaving pores with diameters of 20-90 nm, due to the similarity in atomically exposed π-π bonds in graphene and carbon nanotubes. Thus, peptides that bind to either the edge or planar surface can be identified and used in site-specific functionalization of graphene-based devices. Gaining a deeper understanding of the mechanisms of these interactions requires computational modeling.

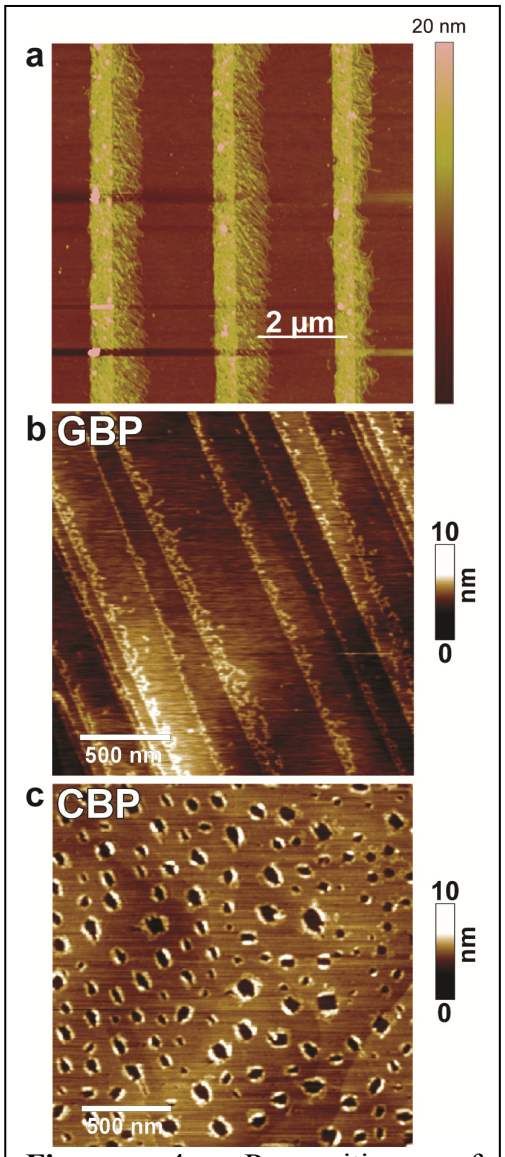


**Figure 4.** Recognition of graphene. (a) AFM image of graphene NWs following incubation with the graphene-binding phage GBP. (b) AFM image obtained from graphene surface exposed to GBP peptide. (c) AFM image of a graphene surface exposed to CBP peptide assembled onto the graphene plane and the graphene nanostrip (GNS).

## D. Molecular Modeling

Theoretical modeling plays an important role in elucidating: 1) structures of peptides and their interaction with target analytes, 2) the interaction of peptides with nanomaterials, and 3) mechanisms of sensor response. In some instances, our calculations reveal that acid/base binding equilibria among the peptides and odorant compounds are significant factors in achieving selectivity. For example, the DLESFLD ammonia-binding peptide used in our SiNW studies[32] forms an interesting structure in which the non-polar groups (leucines and phenylalanine) stack on one side of the peptide and the polar groups (aspartic and glutamic acids, serine) line up on the other (Fig. 5a). It was found that ammonia reacts favorably (-7.4 kcal/mol) with the N-terminal aspartic acid residue (ASP) to form a unique hydrogen-bond center at the terminal acid and amine groups. By contrast, the reaction of acetic acid with this peptide has a near-zero enthalpy of reaction (+0.4 kcal/mol), and the ratio of protonated to deprotonated peptide is 1:3 at room temperature.

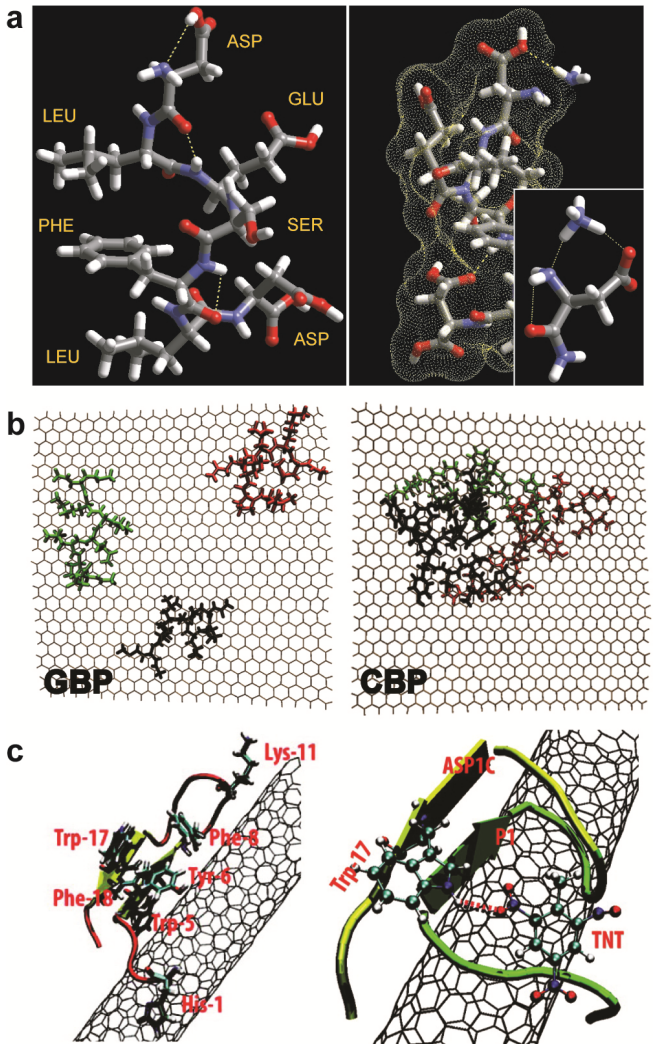


**Figure 5.** (a) The left panel shows the lowest conformation of the ammonia binding peptide. The polar and non-polar amino acids align on opposing sides. The right panel shows ammonia binding at the neutral N-terminus ASP. (Inset) Ammonium stabilized by hydrogen bonds to the deprotonated aspartic acid and the N-terminus. (b) The lowest energy conformations of GBP (left) and CBP (right) on a 5 nm × 5 nm model graphene, obtained from molecular dynamics simulations. (c) P1ASP1C peptide structure prediction in the presence of SWNT using molecular dynamics simulations (left). Modeling predicts that TNT binds to the P1ASP1C–SWNT hybrid via a H bond with Trp[17] and π-π interaction with the SWNT surface (right).

For peptide binding to graphene, to further elucidate the complementary binding properties of edge-selective GBP and plane-selective CBP, extensive molecular dynamics (MD) simulations were performed on the peptide-graphene complex. The peptides GBP and CBP were pre-equilibrated in TIP3P water and randomly positioned above the plane center, near the zigzag edge, or near the armchair edge of a ~5 nm × 5 nm graphene model. Figure 5b shows the most probable conformation of GBP or CBP on graphene after 20 ns equilibration. GBP localizes to within 1.5 nm distance from the graphene edge with weaker interaction energy (-109 kcal/mol), while CBP resides close to the graphene center with stronger interaction energy (-148 kcal/mol). Our computational study clearly indicates that the CBP peptide extends its aromatic amino acid residues -H-Y-W-Y-F- to maximize the ring-ring off-stack π-π interactions[33] between the peptide and graphene surface as previously described,[23] whereas GBP is electrostatically

attracted to the hydrogen-terminated positive graphene edge via the negatively charged glutamate (E) group.

A bifunctional peptide (named P1-ASP1C) consisting of a SWNT binder (P1) and the ASP1C TNT binder described above was also investigated. First, to investigate the effect of SWNTs on the P1-ASP1C peptide, we obtained the structure of the peptide in its SWNT-bound state using computational modeling. The equilibrated structure is shown in Figure 5c, with a potential energy of about -400 kcal/mol. The final structure shows that the hydrophobic groups $Tyr^4$-$Trp^5$-$Tyr^6$-$Ala^7$-$Phe^8$ from the N-terminal half, and $Trp^{17}$-$Phe^{18}$-$Val^{19}$-$Ile^{20}$ from the C-terminal half pack together to form beta-sheets due to the hydrophobic interaction. Further, we calculated the interaction energy between P1-ASP1C-SWNT and the chemical agents TNT using docking MD simulations. The nitro group of TNT forms a hydrogen bond with $Trp^{17}$ while the ring of TNT stacks on the surface of SWNT. This provides a binding motif for TNT to the P1-ASP1C-SWNT hybrid, where the interaction energy is calculated to be ~9 kcal/mol stronger than TNT with a bare nanotube. Based on these favorable energies, the molecular modeling results appear to validate the concept of using peptides to achieve selectivity in nanosensing devices.

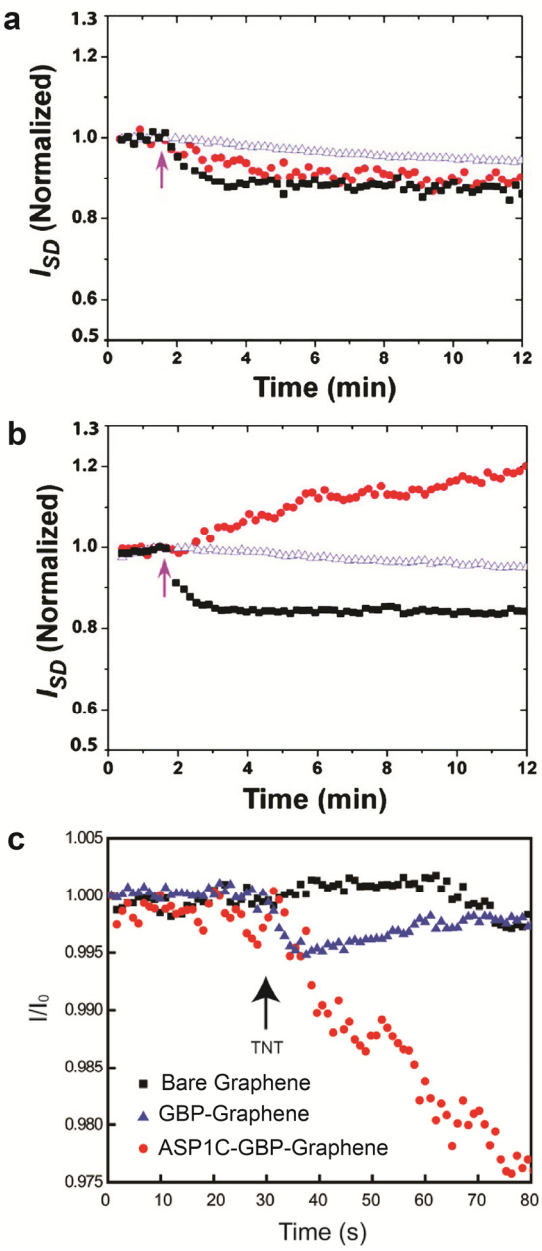


**Figure 6.** Response of (a) bare SWNT and (b) P1ASP1C-coated SWNT to TNT (red circles), RDX (blue triangles), and HPT (black squares). (c) Electrical responses of bare (black), GBP-functionalized (blue) and GBP-ASP1C functionalized (red) graphene sensors to 12 ppb TNT. Arrows indicate the introduction of vapor.

### E. Peptide Nanosensors

The two defining characteristics of sensors are sensitivity and selectivity. While high sensitivities in nanomaterials such as SWNTs, nanowires, and graphene have been repeatedly demonstrated, specificity toward small chemical analytes is a more significant challenge. Further complicating matters is defining what levels of selectivity are adequate. In some cases, ultra-high selectivity is desired – meaning orthogonal off/on (0/1) response characteristics. In other cases, specificity results from combining "broadband" selectivity in individual sensor components with hierarchical arrays to yield a characteristic fingerprint pattern response. Thus, in order to fully elucidate selectivity in peptide nanosensors, responses must be investigated: 1) relative to their bare counterparts (i.e., those which are not functionalized with peptide), 2) relative to the response of the hybrid sensor toward other small molecule analytes, and 3) in the presence of a complex sea of background molecules.

TNT is a well-known chemical explosive, and detection of TNT is critical for

security-related applications. The sensing of ppb levels of TNT by bare and P1-ASP1C peptide coated SWNT-FET devices was investigated by monitoring the drain-source current ($I_{ds}$). The circuit characteristics of these devices are shown in Figures 6a and 6b, respectively. The bare SWNT-FET responds via a decrease in $I_{ds}$, while the P1-ASP1C functionalized SWNT-FET exhibits selective response to 12 ppb TNT via an increase in $I_{ds}$ upon exposure. By contrast, exposure to chemically similar RDX and HPT vapors show equivalent responses in both the bare SWNT-FET and P1-ASP1C functionalized SWNT-FET device, showing non-selectivity toward those small molecules. In other words, P1-ASP1C decorated SWNT-FETs showed a maximal *differential* response to TNT vapor.

Bifunctional designer peptides were also self-assembled onto graphene in order to determine the effect of the immobilized peptide on the activity of a graphene sensor toward TNT. Here, the TNT binding domain was linked to the graphene binding peptide to form GBP-ASP1C. TNT binding onto the graphene surface can take place via direct (non-specific) physisorption, or via selective adsorption at peptide binding sites. To separate these two processes, we included in our sensing experiments a bare graphene sensor and a sensor modified with only GBP as controls. The device was exposed to 12 ppb TNT vapor before and after functionalization with peptide. Figure 6c shows a normalized $I_{ds}$ plot of the result. Clean, bare graphene shows negligible response (< 0.2%) to TNT after 50 s of exposure. Similarly, functionalization with GBP shows only a minor (< 0.3%) response to TNT vapor. Most critically, the GBP-ASP1C functionalized GFET exhibits an order-of-magnitude stronger (~2.5%) decrease in $I_{ds}$ after less than 1 minute of exposure to TNT. These results suggest that the TNT binding domain increases the selectivity and sensitivity of the GFET towards TNT.

Peptide-SiNWs were also characterized as selective sorption-based vapor sensors. The peptide-NW sensors were exposed to target molecules using a flow-through technique. We chose acetic acid and ammonia target molecules for this initial work, because 1) peptide sequences against both have been identified,[16] 2) they are sufficiently reactive to elicit electrical response in the sensors, yet subtle enough for

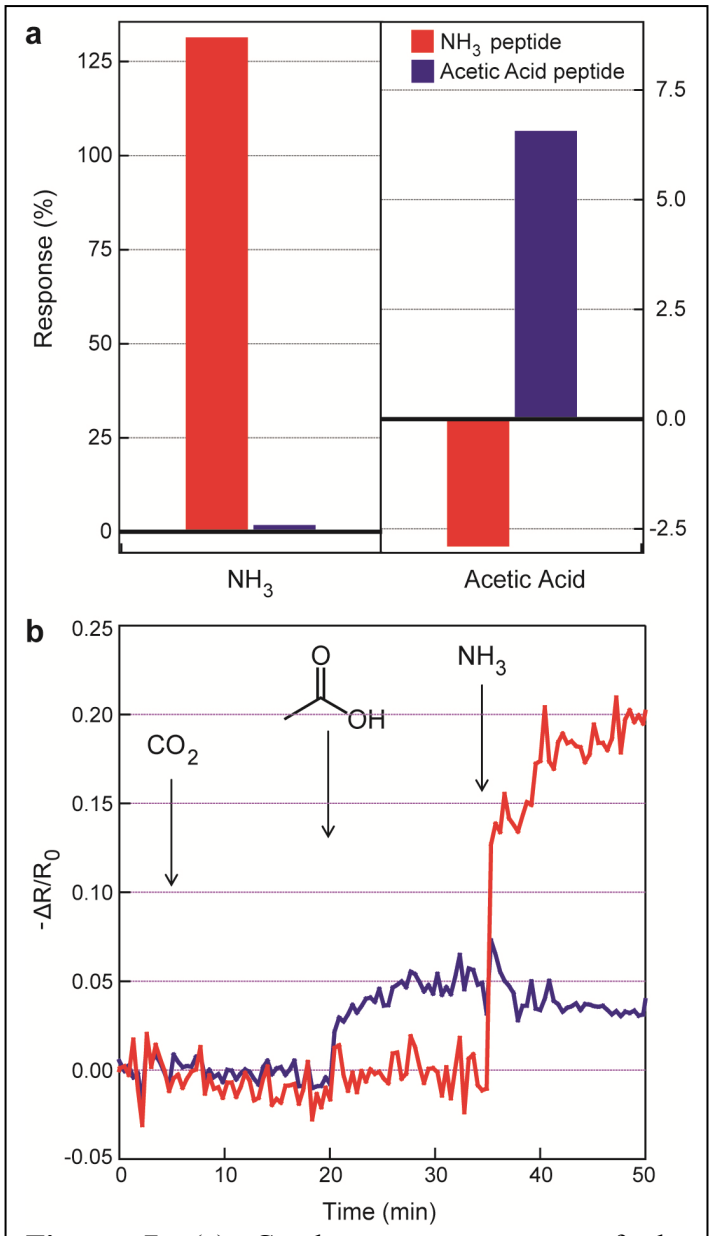


**Figure 7.** (a) Conductance responses of the peptide-nanowire hybrid sensors, averaged over a 5-minute time window of target vapor exposure. (b) Electrical responses of an acetic acid recognition peptide-nanowire sensor (blue), and an ammonia recognition sensor (red) to sequential influxes of 6% $CO_2$, 100 ppm acetic acid, and 100 ppm ammonia, introduced at the times indicated.

exploring the chemical space of peptide recognition sites, and 3) they can serve as exhaled breath disease biomarkers for asthma (acetic acid),[34] and kidney diseases (ammonia).[35] The components comprised one sensor modified with an acetic acid recognition peptide sequence (RVNEWVID),[16] and one with an ammonia recognition sequence (DLESFLD). Normalized sensor responses are shown in Figure 7a. Strikingly, the $NH_3$ recognition peptide displays ca. 75:1 selectivity towards ammonia over acetic acid. This specificity is clearly reversed in the AcOH recognition peptide, with a selectivity ratio of 3.75:1 for the affinity of the acetic acid peptide to AcOH relative to $NH_3$, a value that is in good agreement with previous work.[16]

As a closer approximation towards medical applications, we investigated the performance of our sensors in simulated breath backgrounds. Exhaled human breath contains a mixture of ca. 6% $CO_2$ with hundreds of volatile organic compounds (VOCs), mostly in sub-ppm concentrations, and previous reports have successfully microanalyzed the contents of human breath to identify molecular markers for a range of diseases.[36] Peptide-NW selective sensors on biocompatible plastic substrates provide the potential for implantable, low cost, and continuous monitoring of exhaled breath content at high sensitivities. We tested the responses of peptide-NW hybrid sensors to low levels of $NH_3$ and AcOH molecular markers in a background of 6% $CO_2$. Figure 7b plots the result: injection of AcOH in the $CO_2$ background activates the AcOH peptide, and subsequent exposure of this mixture to $NH_3$ triggers the ammonia targeting device (consistent with Fig. 7a). In addition to affirming molecular specificity, this is a key initial demonstration towards enabling these devices for continuous breath analysis.

**F. Summary**

This report summarizes our initial attempts at addressing some critical questions about the fundamental function and potential applications of hybrid peptide-nanosensors. Peptides represent a happy medium between tapping into the chemical diversity of amino acids – nature's preferred recognition molecules – but without the stability issues associated with proteins. Yet, a significant number of challenges remain, including: 1) identifying larger classes of peptide binders to a host of chemical and biological targets, 2) understanding the mechanisms by which biomolecular interactions between analytes and peptides induce subsequent responses in the nano-transducers, 3) scaling these results from the few-device level to large arrays which can address many targets in parallel, and 4) translating these results from well-controlled laboratory environments to clinical applications and settings. Taken together, these results serve as a model platform in the use of peptide nanosensors for applications ranging from non-invasive breath monitoring to food spoilage or biological and chemical threat detectors.

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**Publications Acknowledging AFOSR Support:**

8. Y. Cui, S. N. Kim, R. R. Naik, M. C. McAlpine. "Biomimetic Peptide Nanosensors." *Acc. Chem. Res.* In Press (2012).

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1. Y. Cui, A. Pattabiraman, B. Lisko, S. C. Collins, M. C. McAlpine. "Recognition of Patterned Molecular Ink with Phage Displayed Peptides." *J. Am. Chem. Soc.* **132**, 1204-1205 (2010).

**Interactions/Transitions:**

**2011 Invited Presentations**

9. "Biomimetic Peptide Nanosensors," AFOSR NMS&E Program Review, 12/6/11 (National Harbor, MD).
8. "Nanotechnology-Enabled Device Interfacing with the Human Body," Drexel University, 11/10/11 (Cherry Hill, NJ).
7. "Nanotechnology-Enabled Device Interfacing with the Human Body," Lockheed Martin Advanced Technology Laboratories, 10/28/11 (Cherry Hill, NJ).
6. "Bionanotechnology-Enabled Multifunctional Sensing," Nanoelectronic Devices for Defense & Security (NANO-DDS) Conference, 9/1/11 (Brooklyn, NY).
5. "Nanotechnology-Enabled Device Interfacing with the Human Body," DuPont Central Research and Development, 6/25/11 (Wilmington, DE).
4. "Nanotechnology-Enabled Device Interfacing with the Human Body," Keynote Speech at Freescale Technology Forum, 6/22/11 (San Antonio, TX).
3. "Nanotechnology-Enabled Interfacing of Devices with the Human Body," Google Tech Talk, 6/6/11 (Mountain View, CA).
2. "Nanotechnology-Enabled Flexible and Multifunctional Sensing," AFOSR Exploring Biological Interfaces Workshop, 4/4/11 (San Juan, Puerto Rico).
1. "Nanotechnology-Enabled Flexible Biomimetic Sensors," AFOSR 2312 DX & EX Program Review, 1/3/11 (National Harbor, MD).

**Air Force Collaborations**

We collaborated closely with Dr. Rajesh Naik and his group at AFRL.

**Transitions**

The results have been used in a project supported by the American Asthma Foundation for selectively detecting molecules which are indicators of asthma in the breath.

**Patents**

1. M. C. McAlpine, M. S. Mannoor. Broadband Detection of Bacteria Using Antimicrobial Peptides Immobilized on an Electrical Sensing Device. U.S. Patent Pending 13/171,120.

**2011 Honors/Awards**

1. National Academy of Engineering – Frontiers of Engineering

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